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Biodegradation Studies of Carboxymethyl Tartronate

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Biodegradation Studies of Carboxymethyl Tartronate

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Once acclimated to CMT the biomass could be starved in regard to this substrate for at least 1.6 times the sludge age and still retain capacity to degrade the material upon re-introduction.

Activated carbon was not found to be effective for CMT removal at a pH value of 7.

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BIODEGRADATION STUDIES OF CARBOXYMETHYL TARTRONATE

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

This report describes biodegradation studies conducted on the chemical carboxymethyl tartronate, which has been considered as a replacement for phosphates in laundry detergents.

Francis T. Mayo
Director
Municipal Environmental Research
Laboratory

ABSTRACT

Carboxymethyl tartronate (CMT) was shown to be biodegradable in bench scale activated sludge reactors.

After initial exposure to CMT in continuous flow systems an acclimation period of 14 weeks was necessary before efficient degradation occurred.

Once acclimated to CMT the biomass could be starved in regard to this substrate for at least 1.6 times the sludge age and still retain capacity to degrade the material upon re-introduction.

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Mr. Timothy Miller, University of Cincinnati, aided in the project during the summer of 1976.

Mr. Robert Bloomhuff, Municipal Environmental Research Laboratory, provided analytical assistance.

Mrs. Rita Bender, Municipal Environmental Research Laboratory, prepared the manuscript for final publication.

SECTION I

INTRODUCTION

In recent years, the national concern for protecting natural waters from environmental pollution has been extended to the problem of accelerated eutrophication due to man's activities. Although the basic understanding of all causes of eutrophication is not fully agreed upon, it is certain that compounds of nitrogen or phosphorus can contribute significantly to the problem.

In the case of phosphorus compounds it is recognized that about half the phosphorus content of municipal wastewater is derived from phosphate containing detergents. Since municipal wastewaters are discharged as a point source, control of effluent phosphorus compounds could be achieved by banning phosphates in detergents, or by modified process control at the wastewater facility. In view of the low residual phosphorus required for eutrophication control it is likely both approaches will be implemented (1).

Phosphates in synthetic detergent formulations serve as water softeners, therefore any material that would chelate calcium ions can be considered as a replacement for phosphates. Various organic chelating agents have been evaluated by Pollard (2). Bunch and Ettinger (3) studied the biodegradation of a series of organic chelating agents using the Bunch and Chambers test procedure (4). For various reasons none of the compounds studied by these investigators gained acceptance as a commercial replacement for phosphates in the United States.

It may be reasonably assumed that current regulatory attitudes and past experience by detergent manufacturers will dictate that neither toxic or nondegradable materials will be introduced into a detergent product. Recently attention has been directed towards synthetic, nitrogen free, ether polycarboxylates as phosphate replacements for use in areas where phosphates have been restricted. A material of this type has been synthesized by Lamberti (5). The material is trisodium carboxymethyl oxysuccinate (CMOS). Biodegradation and other environmental tests are currently being conducted by the manufacturer. Another compound of this class, trisodium carboxymethyl tartronate (CMT) has been investigated by Gledhill (6). Using continuous and semi-continuous activated sludge processes he reported that CMT was biodegradable after a suitable acclimation period for the microorganisms.

Trade journals indicated that this latter compound would be vigorously promoted and production could reach 70 million kilograms annually by the year 1978 (6, 7).

It can be estimated that municipal wastewater would contain CMT at a concentration of 20 mg/l if this material replaced all the phosphate in today's detergent formulations.

In anticipation of this product's wide scale distribution, it was deemed important that the Municipal Environmental Research Laboratory confirm the biodegradability of CMT.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

As a result of this research these conclusions have been reached:

1. Trisodium 20-oxa-1, 1, 3-propane tricarboxylate (carboxymethyl tartronate or CMT) is biodegradable by activated sludge treatment.

2. An acclimation period of 14 weeks is required before the biomass develops a population capable of efficient degradation.

3. External innoculum of soil and municipal wastewater microorganisms into a synthetic medium did not reduce the acclimation period.

4. Once acclimated the biomass retained the capacity to degrade CMT for at least 1.6 times the sludge age in the absence of the substrate.

5. Activated carbon was efficient in adsorption of CMT from distilled water at pH 3. At pH values of 7 and 9 carbon adsorption was not effective.

6. The synthetic wastewater employed in these studies gave similar results when compared with municipal wastewater.

RECOMMENDATION

In view of the above the following recommendation is made:

1. The degradation of CMT in anaerobic sludge digestion systems should be investigated.

SECTION 3

MATERIALS AND MEHODS

Chemistry and Characteristics of Carboxymethyl Tartronate (CMT)

CMT can be described as trisodium 2-oxa-1, 3-propane tricarboxylate in the international nomenclature system. This chemical is the major ingredient in a formulation named "Builder M" which is combined with synthetic surfactants to produce a complete laundry product.

"Builder M" functions to sequester calcium and magnesium ions in aqueous solution. It is a buffering agent, has deflocculating properties, is compatible with other ingredients such as bleaches, and is stable in neutral and basic solution over a wide range of temperature. The material is not stable in strong acid solution.

Commercial lots of "Builder M" are expected to contain mixtures of several polycarboxylates and other ingredients. A typical formulation would have the composition shown on Table 1 (8). For the work described in this report Lot #237028 "Builder M" was used. It had a CMT content of 78 percent. Throughout the report this material will be identified as CMT.

Biodegradation Apparatus

Bench-scale Bio-oxidation Units:

One portion of the biodegradation studies on CMT was conducted with aerobic suspended growth reactors of 5.7ℓ capacity constructed as described by Ludzack (9). This continuous flow type unit is shown on Figure 1.

The feed solution for the reactors was placed in 20ℓ capacity polyethylene containers and stored in a refrigerator maintained at 5°C to retard microbial action. Mixing, to prevent settling of the feed solution, was accomplished by low rate air addition through a coarse bubble sparger located in the containers. Chemical metering diaphragm pumps transferred the feed solution from refrigerated storage to the reactors maintained at room temperature. Effluent from the reactors were collected in 20ℓ capacity glass bottles.

300 ml Bio-oxidation Units:

Continuous flow studies were also performed with 300 ml aerobic reactors shown on Figure 2. Feed solution for these reactors was contained in 4ℓ

glass bottles. Refrigeration was not provided. Mixing of the feed was accomplished by magnetic stirrers. A Technicon Auto Analyzer manifold pump was used to transfer the feed to the small reactors through the side inlet to the aeration chamber. Reactor effluent was collected in 4ℓ glass bottles. These reactors were maintained at room temperature.

Biomass Development:

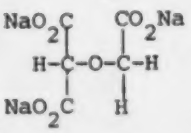
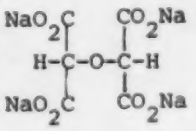
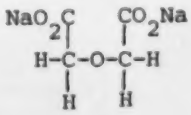
Initial studies on CMT degradation were conducted with the 5.7ℓ of mixed liquor from an activated sludge pilot plant treating municipal wastewater were placed in the reactor. Synthetic wastewater, on a continuous flow basis, was then started and the reactors were operated in this fashion for one month before actual experimentation on CMT.

Throughout the entire study, sludge was wasted from the reactors to maintain the biomass at a concentration of about 2,500 mg/l on a volatile solids basis.

Feed Solutions

At different times during the study the feed to the bio-oxidation reactors was either municipal wastewater or a synthetic waste prepared from laboratory reagents.

TABLE 1. COMPOSITION OF BUILDER M

Name of Ingredient	Chemical Structure	Percent of Total Product (Weight Basis)
Carboxymethyl tartronate (CMT)		78
Ditartronate		5
Diglycolate		3
Sodium carbonate	Na_2CO_3	6
Water of Hydration	H_2O	8

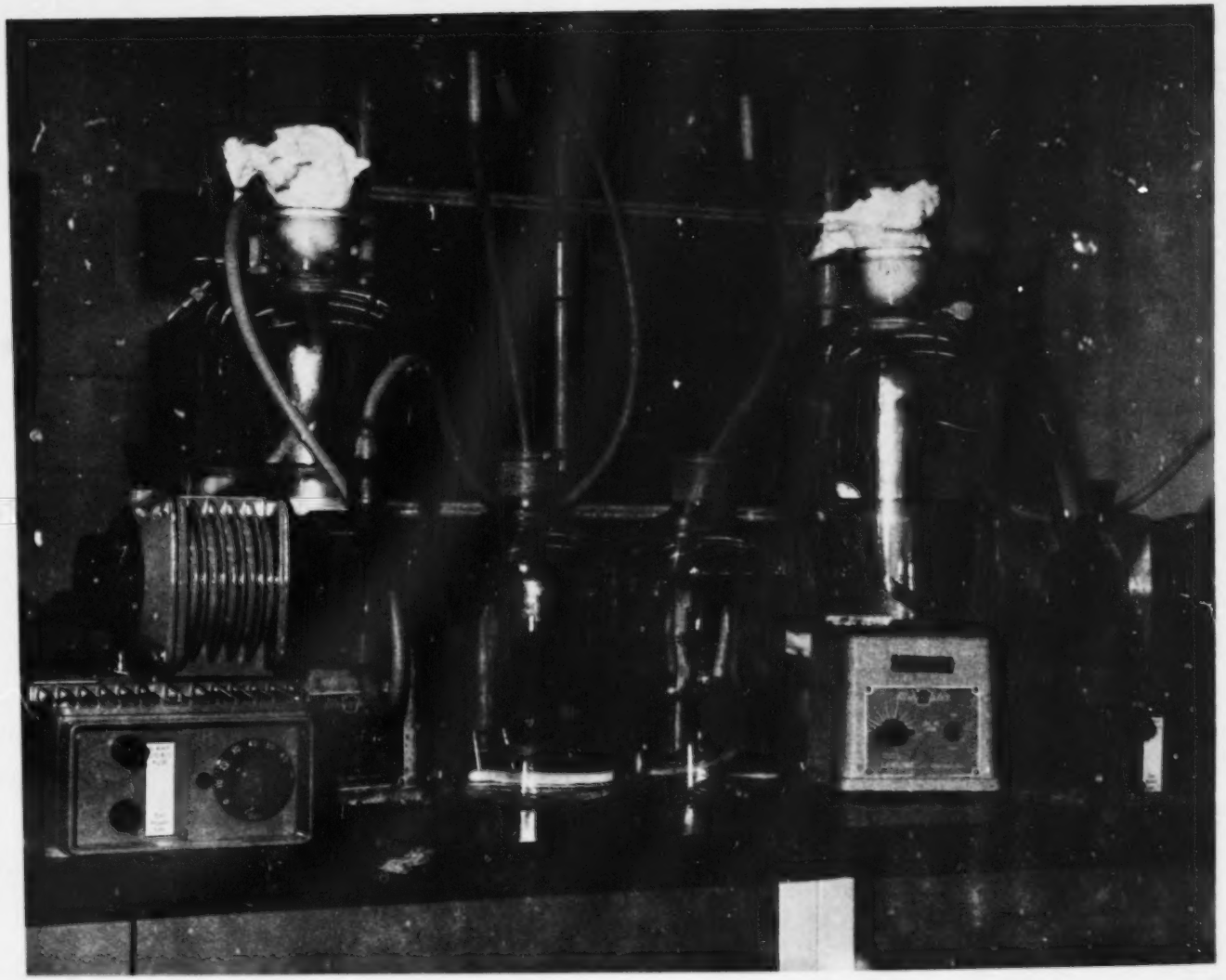


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FIGURE 1. 5.7 Liter Suspended Growth Reactors

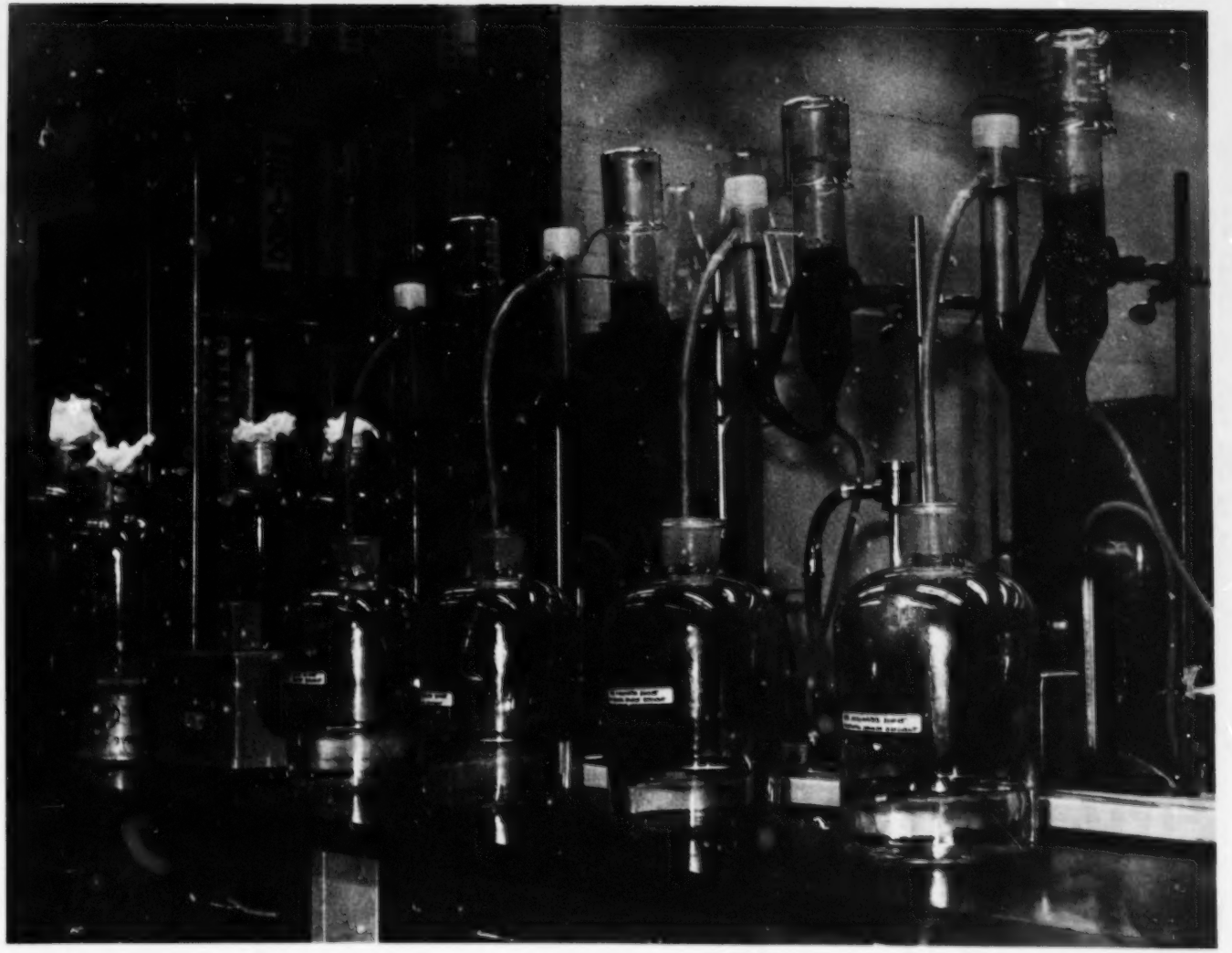

FIGURE 2. 300 ml Suspended Growth Reactors

Municipal Wastewater:

When studying the biodegradation of CMT with municipal wastewater feed the source of wastewater was the Wilmer Avenue interceptor sewage of the City of Cincinnati. The collected raw wastewater was settled for 0.5 hr and the supernatant filtered through a layer of glass wool before transfer to the dosing container. Each second day the dosing containers were thoroughly washed before being returned to service.

Synthetic Waste:

In order to eliminate the variable nature of municipal wastewater and prevent possible introduction of toxic materials during the acclimation period a synthetic waste was used in most of these studies. This feed solution was formulated after a review of prior published formulations (10 through 23).

The final synthetic waste solution, prepared by diluting stock solutions with distilled water had the following composition:

Constituent	Concentration, mg/l
KH_2PO_4	8.5
K_2HPO_4	22
Na_2HPO_4	33
NH_4Cl	2
$MgSO_4$	22
$CaCl_2$	36
$FeCl_3$	0.3
Urea	50
$NaHCO_3$	300
Yeast Extract (Difco)	55
Baco Peptone (Difco)	50
Meat Extract (Difco)	50
Fish Meal Extract (Purina Trout Chow)	0.5 ml

The fish meal extract was prepared by grinding 10g of Purina Trout Chow with 200 ml of distrilled water in a high speed blender for 3 min. The mixture was allowed to settle for 10 min and then 0.5 ml added to each liter of synthetic waste. Experience has shown this additive helps control bulking of sludges produced from synthetic feeds.

The synthetic feed typically has the following characteristics:

Item	Concentration, mg/l
Chemical oxygen demand	160
Total organic carbon	76
Suspended solids	2
Total Kjeldahl nitrogen	47
Ammonia nitrogen	2
Nitrite and nitrate nitrogen	<0.1
Total phosphorus	13
Alkalinity, as calcium carbonate	215
pH	7.3 units

The nitrogen and phosphorus content is slightly higher than most municipal wastewaters. However it was deemed necessary to have these nutrients in excess to insure the waste was not growth limiting. The suspended solids are low because the waste is composed of soluble materials for ease of preparation of the feed from stock solutions.

Special Inocula

To insure that an acclimated biomass could be developed, on occasion a soil extract or wastewater concentrate was added to the reactors to provide a diversity of microorganisms.

Soil Extract:

To prepare a soil extract 50g of forest soil and 50g of garden soil; both collected in Anderson Township, Ohio, were added to 1ℓ of distilled water and blended at high speed for 1 min. The blend was transferred to an Erlenmeyer fask and shaken on a rotary shaker for 15 min. The mixture was then allowed to settle for 0.5 hr. The resulting supernatant was used to inoculate the reactors to yield 2 percent by volume of the mixed liquor.

Wastewater Concentrate:

Wastewater from the Wilmer Avenue interceptor sewer of the City of Cincinnati was placed in a refrigerator and allowed to settle for six hours. The supernatant was discarded and the settled solids were used to inoculate the reactors to yield 2 percent by volume of the mixed liquor.

General Analytical Methods

Except as noted all methods were in accordance with the procedures authorized in "Standard Methods for the Examination of Water and Wastewater," 13th Edition (24). The tabulation below lists the various tests and procedures used according to the cited pages of Standard Methods.

Test	Procedure	Page Number
pH	Glass electrode	276-281
Temperature	Mercury thermometer	348-349
Alkalinity	Potentiometric	52-55
Suspended solids	224C	537-538
Total solids	224A	535
Volatile suspended solids	224D	538
Volatile total solids	224B	536
Chemical oxygen demand	Dichromate reflux	495-499
Kjeldahl nitrogen	Digestion	469
Ammonia nitrogen	Automated	616-620*
Oxidized nitrogen	Automated	620-624*
Phosphorus, total	Automated	624-628*

* 14th Edition of Standard Methods

Analytical Methods for CMT

Two methods were used to determine CMT. A colorimetric method, which is simple and fast but non-specific and therefore subject to interferences, was employed for daily monitoring of CMT concentrations. A gas chromatographic method which is specific but time consuming and cumbersome was used periodically to check the colorimetric results.

All analytical results in this report are based on using Lot #237028 of "Builder M" as 100 percent CMT content; since this single lot was used throughout the study for both reference and working samples.

Colorimetric Method:

Except for minor modifications the colorimetric method is the same as that reported by Viccaro and Ambye for the analysis of carboxymethyloxysuccinate (25).

The method is based on the hydrolysis of CMT to glycolic acid, which in turn yields formaldehyde in the presence of high concentrations of sulfuric acid. β-naphthol is added to condense with the formaldehyde to form a highly colored diarylamine. The intensity of the color is proportional to the concentration of CMT in the original sample. A detailed description of the method is given in Appendix A, Part I.

A thorough shakedown of the method was made before the biodegradation studies were initiated. Samples of distilled water, tap water, secondary effluent and raw wastewater were dosed with quantities of CMT up to 20 mg/l. Linear and consistent responses were obtained on all samples except CMT in distilled water. This unusual problem was circumvented by using tap water for reference samples. The erratic results with distilled water are probably due to the lack of ions to stabilize the colored end product of the reaction. As with many colorimetric procedures occasional aberrant results were experienced. Minor fluctuations of CMT content of duplicate samples raised doubts about the reliability of the method. A thorough test of the method was made in September 1976. A total of 34 samples of standards and test samples were assayed independently by both MERL and Monsanto Chemical Company personnel. The collective results agreed well and indicated the procedure was suitable for daily monitoring.

Samples of raw wastewater and final effluents containing no added CMT seldom exceeded 2 and 1 mg/l respectively. This background level is probably due to materials such as glycolic acid and acetaldehyde which can be expected in low concentrations in wastewaters.

Gas Chromatographic Method:

The gas chromatographic method used in this study was developed by C. B. Warren of Monsanto Chemical Company and has not been published. It is based on esterification of CMT with n-propyl alcohol. A detailed description is given in Appendix A, Part II.

Experiments with raw wastewater and secondary effluent dosed with CMT at different concentrations produced linear response in peak height. However plots of detector response versus CMT concentration shifted with different samples, although no interferring substances were noted in any wastewater or effluents. Also duplicate samples of a CMT standard solution occasionally produced inconsistent results. These anomalies are suspected to be due to incomplete esterification of CMT.

Since both the colimetric and gas chromatographic procedures were shown to have inherent limitations, the fate of CMT in these biodegradation studies was not judged by any single analysis but rather by establishing a pattern of results and comparison of the results obtained by each procedure.

SECTION 4

RESULTS

5.7 Liter Continuous Flow Reactor Studies

The biodegradation of CMT at a concentration of 20 mg/l in synthetic feed was studied with and without inoculum of soil extract and wastewater concentrate. In each case a control reactor, without CMT addition was operated under similar conditions as the experimental reactors.

Synthetic Feed Only:

Table 2 gives the operational characteristics and efficiency for the experimental and control reactors during this portion of the study, which covered a period of 23 weeks. The values for CMT are not given because an acclimation period for degradation was required and several spike doses were added; therefore, average values would be meaningless. The data on Table 2 shows the reactors were operated under a control scheme that was favorable to oxidation of COD, TOC and organic nitrogen. Almost all the oxidized nitrogen was in the nitrate form.

Values for nitrite in the effluents never exceed 2 mg/l. The control and CMT experimental reactor produced nearly identical quality effluent indicating no adverse effect of the 20 mg/l CMT dose on overall efficiency.

Figure 3 shows the CMT concentration of the effluent from each reactor on a weekly average basis for the entire study period. Also shown is the TOC concentration of the CMT reactor effluent. The control reactor's effluent TOC values were slightly lower during the early portion of the study and slightly higher during the later portion. As shown on Table 2 the average TOC values for both effluents, over the entire study, were equivalent.

The figure shows that the control effluent gave small but measurable amounts of CMT when using the colorimetric procedure due to the non-specific nature of the assay. Starting at week #1 with the first introduction of the 20 mg/l CMT feed the CMT concentration of the experimental reactor effluent increased greatly. For a period of 12 weeks the CMT content of the effluent was erratic. The CMT concentration did not approach the control values until week #14 and then stabilized to near background levels for four weeks. At that time the feed CMT concentration was increased to 50 mg/l for a period of two weeks. The reactor degraded the increased concentration of CMT to background levels. The feed concentration was then increased to 100 mg/l CMT for a period of two weeks. After an initial slight rise in

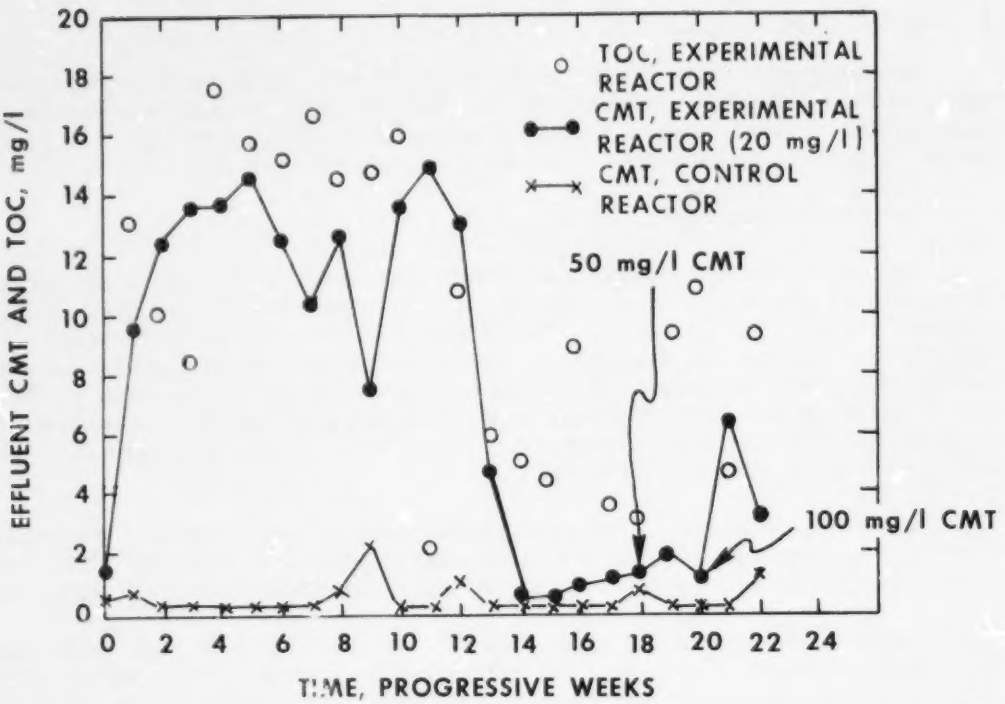

FIGURE 3. CMT and TOC of Reactor Effluents with Synthetic Feed

TABLE 2. Efficiency and Operational Data for Synthetic Feed Reactors for a Period of 23 Weeks

Efficiency Items	Reactor Average and Range			
	Control		CMT Experimental	
	Feed	Effluent	Feed	Effluent
COD, mg/l	161 148-175	20 11-32	159 151-175	22 12-29
TOC, mg/l	79 70-88	9 3-16	79 69-89	10 2-17
SS, mg/l	2 1-11	3 1-8	2 1-8	3 1-10
NH_4-N	3 1-4	1 0.1-9	2 1-3	1 0.1-9
Oxidized-N, mg/l	0.1 0.1-0.4	33 14-51	0.3 0.1-1.8	33 24-53
TKN, mg/l	41 34-48	2 0.2-10	41 35-48	2 0.2-10
Total-P	13 11-15	13 9-24	13 11-15	13 8-23
Alkalinity, mg/l as $CaCO_3$	180 102-243	56 14-80	186 144-245	53 13-86

Operational Items	Control Reactor	CMT Experimental Reactor
pH, units	--- 6.9-7.3	--- 6.9-7.3
DO, mg/l	3.8 2.4-5.0	3.7 2.8-4.2
Temperature, C	20.6 20.0-21.5	20.8 20.0-21.5
MLSS, mg/l	3,200 3,000-3,600	3,300 2,900-3,700
MLVSS, mg/l	2,100 1,900-2,400	2,200 1,900-2,700
Volatile, Percent	66 61-72	67 62-75
F/M, g COD/g MLVSS/d	0.29 0.20-0.36	0.28 0.21-0.35
SRT, days	17 15-18	17 15-18
Hydraulic detention time, hr	6	6

effluent CMT the experimental reactor's effluent CMT concentration began to decline toward background concentrations.

It was concluded that an activated sludge biomass developed with the synthetic wastewater, without an external inoculum of microorganisms, could acclimate to CMT and efficiently degrade the material. The on-set of acclimation however did not occur until 12 to 13 weeks of continuous exposure to CMT. Once acclimation occurred the biomass could efficiently degrade increased concentrations of the material.

Synthetic Feed With Soil Extract and Wastewater Concentrate as Inoculum:

To determine if the long acclimation period observed in the previous continuous flow study could be shortened, by adding an external source of microorganisms to enhance population diversity, an additional study was performed.

Two reactors, one a control and the other receiving 20 mg/l of CMT, were operated in the same manner as the previous study. Each 48 hours the continuous flow reactors were dosed with soil extract and wastewater concentrate. Each inoculum was added to replace 2 percent of the reactor volume after the reactors had remained quiescent for one half hour and the proper amount of supernatant withdrawn.

This study was carried out for 18 weeks. Table 3 gives the operational characteristics and efficiency for the experimental and control reactors during this portion of the study. In regard to feed concentrations and efficiency, the results were quite similar to the 23-week study with synthetic feed alone. The only major difference was the level of MLSS in the reactors and the percent volatile content of this biomass. Due to the addition of the soil extract and wastewater concentrate inert solids were added to the system, decreasing the volatile content from about 66 percent to 44 percent.

Figure 4 shows the CMT concentration of the effluent from each reactor on a weekly average basis for the entire study period. Also shown is the TOC concentration of the CMT reactor effluent. The control reactor's effluent TOC values were very similar to the experimental effluent values. As shown on Table 3 the average TOC values for both effluents, over the entire study, were about the same. Also the effluent TOC values are similar to those on Table 2 indicating the external inoculum procedure did not impose a significant increase in organic loading on the reactors.

The pattern of CMT concentration in the experimental reactor effluent was not very different than the pattern observed on Figure 3. After a rapid increase at the start of the 20 mg/l CMT feeding there was erratic response followed by a declining concentration. At week #14 the effluent concentration of the CMT experimental reactor stabilized to the background levels of CMT in the control reactor effluent.

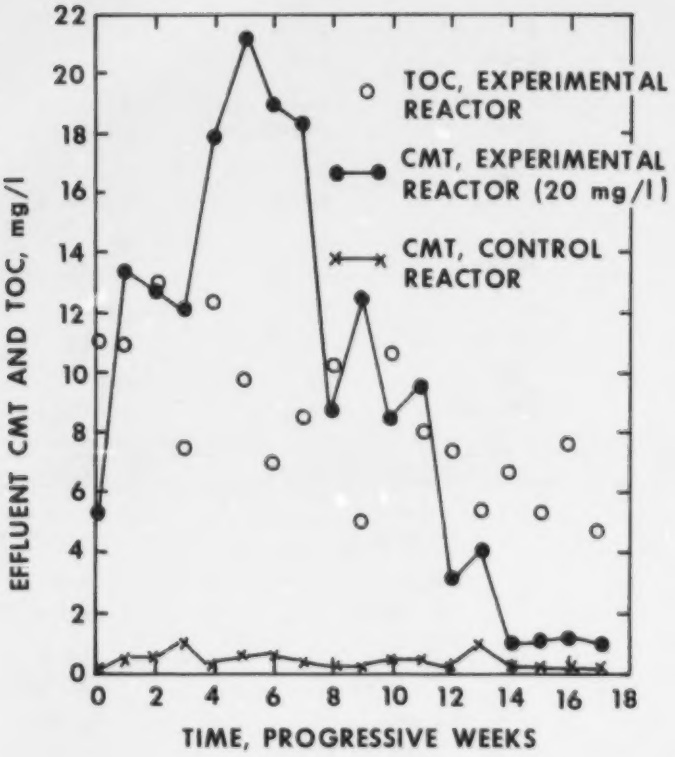

FIGURE 4. CMT and TOC of Reactor Effluents with Synthetic Feed and External Inocula

TABLE 3. Efficiency and Operational Data for Synthetic Feed and External Inoculum Reactors for a Period of 18 Weeks

Efficiency Items	Reactor Average and Range			
	Control		CMT Experimental	
	Feed	Effluent	Feed	Effluent
COD, mg/l	160 148-176	16 10-29	157 151-164	19 14-29
TOC, mg/l	79 70-87	6 3-14	79 69-84	8 5-13
SS, mg/l	2 1-10	2 1-3	2 1-8	2 1-3
NH_4-N	2 1-3	0.1 0.1-0.3	2 1-3	0.1 0.1-0.3
Oxidized-N, mg/l	0.1 0.1-0.4	37 23-59	0.2 0.1-0.4	34 25-39
TKN, mg/l	42 34-48	1 0.1-2	40 35-45	1 0.1-2
TP, mg/l	13 11-14	13 10-15	13 11-14	12 10-15
Alkalinity, mg/l as $CaCO_3$	190 171-243	54 30-83	189 165-245	57 34-76

Operational Items	Control Reactor	CMT Experimental Reactor
pH, units	--- 6.9-7.2	--- 6.9-7.3
DO, mg/l	4.0 3.0-5.6	3.8 3.0-5.0
Temperature, C	20.9 20.0-21.5	20.7 20.5-21.5
MLSS, mg/l	6,600 3,400-7,200	6,600 3,200-7,300
MLVSS, mg/l	2,900 2,100-3,200	2,900 2,100-3,800
Volatile, Percent	44 37-68	44 37-67
F/M, g COD/g MLVSS/d	0.22 0.19-0.28	0.22 0.17-0.33
SRT, days	18 15-19	19 18-19
Hydraulic detention time, hr	6	6

It was concluded that external inoculum of soil and wastewater microorganisms did not accelerate the acclimation time of the reactor biomass to CMT degradation. CMT is a compound that requires adaptive enzyme formation. Since the external inoculum furnished a source of heterogenous microorganisms, and the acclimation time was not shortened, apparently the adaptive enzyme system for CMT degradation is not a common occurrence.

300 ml Continuous Flow Reactor Studies

To determine if the synthetic feed studies were as suitable as municipal wastewater for biodegradation studies of this nature, and to observe the degradation of higher concentrations of CMT, four small 300 ml reactors were used.

One unit was fed continuously with synthetic feed containing 50 mg/l CMT and the other was fed continuously with settled municipal wastewater dosed with 50 mg/l CMT. For each type feed a control reactor was operated. The control feeds contained no CMT. The mixed liquor content of each reactor was obtained from the CMT acclimated biomass developed in the 5.7 l reactor study reported in the above section. The reactors were operated to maintain about 2,000 mg/l of MLVSS in each reactor. The F/M ratio was about 0.25 g COD/g MLVSS/d in each reactor. The hydraulic detention time was 6 hr. Duration of the study was three weeks. Results are shown on Table 4. These data show that there was no difference in the efficiency of biodegradation of CMT when this substrate was in either synthetic or municipal wastewater, and a CMT concentration of 50 mg/l could be degraded.

The percent loss of CMT was calculated on actual effluent CMT values, neglecting the background level of CMT in the control reactors effluents. Since the CMT colorimetric assay is non-specific and variable, and the concentrations observed in the effluents were low compared to feed concentrations, use of a corrected value would have no significant effect on the efficiency values.

CMT Starvation to Determine Acclimation Stability

To determine if the induced enzyme system of CMT acclimated biomass was a stable property two 300-ml reactors were used. The mixed liquor for each reactor was acclimated biomass from the 20 mg/l CMT study utilizing the 5.7 l continuous flow reactors. One small reactor received synthetic feed containing no CMT and the other received municipal wastewater containing no CMT. These units were operated continuously on their respective CMT free feeds for 28 days under operational parameters listed in Table 2.

At the end of this CMT starvation period CMT at a concentration of 20 mg/l was reintroduced into each type feed. Table 5 shows CMT concentrations for feeds and effluents for both type wastewaters and percent loss of CMT for two weeks prior to CMT starvation, during the four-week starvation period and after resumption of CMT dosing.

TABLE 4. Degradation of CMT in Synthetic and Municipal Wastewater Using 300 ml Reactors

Reactor	CMT, mg/l Progressive days								
	1	4	6	8	11	13	15	18	20
Synthetic Wastewater, 50 mg/l CMT									
Feed	39	52	54	49	44	52	48	47	53
Effluent	1.7	3.7	4.5	3.7	2.8	3.2	2.8	2.6	3.5
Percent Loss of CMT*	96	93	92	92	94	94	94	94	93
Municipal Wastewater, 50 mg/l CMT									
Feed	45	50	55	50	52	53	48	49	51
Effluent	3.1	3.4	2.7	2.4	2.3	2.6	3.2	1.9	3.1
Percent Loss of CMT**	93	93	95	95	96	95	93	96	94

* Not corrected for background level of CMT in a synthetic wastewater control effluent which averaged 0.3 mg/l during this study.

**Not corrected for background level of CMT in a municipal wastewater control effluent which averaged 0.8 mg/l during this study.

Immediately upon re-introduction of CMT into each type feed the response of the previously acclimated biomass in each reactor was rapid. Very efficient degradation of CMT occurred during the first 24-hour period.

This was not an extreme test of adaptive stability since the sludge retention time was about 17 days. The 4-week starvation period was only 1.6 times the sludge retention period and consequently a portion of the original mixed liquor still existed in the reactors at the end of this time. The studies did show that acclimated microorganisms could be deprived of CMT for a considerable length of time and still retain the capacity to degrade the substance upon subsequent exposure.

Carbon Adsorption Study

During the initial phase of the biological studies when it was not clearly evident that CMT was biodegradable a preliminary screening for carbon adsorption was conducted.

Various dosages of activated carbon were added to 20 mg/l distilled water solutions of CMT. Adsorptivity at pH 3, 7 and 9 was investigated.

TABLE 5. Stability of Acclimated Biomass for Biodegradation of CMT

Reactor	Pre-starvation Continuous feed, 20 mg/l CMT	Starvation Continuous feed, no CMT added	Re-start CMT Continuous feed 20 mg/l CMT			
	CMT, mg/l					
	2 Week Average	4 Week Average	Progressive days 1	2	7	14
Synthetic Wastewater						
Feed	21	1.1	20	20	20	20
Effluent	1.3	1.1	1.4	0.5	1.4	0.9
Percent Loss of CMT*	94	---	93	97	93	96
Municipal Wastewater						
Feed	20	1.3	20	20	20	20
Effluent	1.7	0.6	2.8	2.5	1.7	1.8
Percent Loss of CMT*	92	---	86	87	92	91

*Not corrected for background CMT values.

The solution at pH 3 showed over 90 percent removal of CMT at the various carbon dosages. At pH values of 7 and 9 the carbon adsorption was not efficient, and even at high carbon dosages the removal of CMT did not exceed 50 percent.

Detailed data is given in Appendix B.

SECTION 5

DISCUSSION

These studies on CMT biodegradation were conducted to reflect usual operating parameters controlled in activated sludge treatment. Under this set of conditions CMT was shown to be biodegradable; however, it was determined that a rather protracted induction period was necessary before the adaptive enzyme system for efficient oxidation was obtained.

Once acclimation of the biomass occurred, the stability of the system was compatible with the expected occurrence of CMT in municipal wastewater. Acclimation was shown to be retained for a period of 1.6 times the SRT of the biomass. If CMT were introduced into municipal wastewater as a substitute for phosphates in detergent formulations, the material would occur as a continuous constituent of wastewater. Once the municipal biological system acclimated to the presence of CMT a continual high efficiency of degradation would be expected.

The formulation of synthetic wastewater used in these studies was shown to be a suitable substitute for determination of biodegradation in comparison with municipal wastewater.

The pattern of adsorption of CMT by activated carbon at the several pH values is consistent with the chemical structure shown in Table 1. At pH 3 the compound would be in the unionized carboxylic acid form and have greater affinity for carbon adsorption than when it is ionized at the more alkaline pH values.

Since municipal wastewaters usually have pH values near 7 the use of activated carbon for removal of CMT would not be practical.

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APPENDIX A

ANALYTICAL METHODS

Two analytical methods were used to monitor CMT (also referred to as "Builder M"); a colorimetric method which is simple and fast, but non-specific and subject to minor interferences; and a gas chromatographic (GC) method which is specific, but time consuming and cumbersome. For daily monitoring of the fate of CMT the colorimetric method was used exclusively. Once a week, however, samples were also analyzed gas chromatographically for comparison of results by both methods.

The "Builder M" used in this investigation contained only 78 percent CMT; however, all calculations were based on 100 percent purity.

Colorimetric Method

Except for minor modifications this method is the same as one reported by Viccaro and Ambye (25) for the analysis of carboxymethyloxysuccinate. This method is based on the hydrolysis of CMT to produce glycolic acid, which in turn yields formaldehyde in the presence of high concentrations of sulfuric acid. β-naphthol condenses with formaldehyde to form highly colored diarylamines and the intensity is proportional to the quantity of CMT in the sample. A detailed description of the method is given later in Part I of this Appendix.

A thorough shakedown of the method was made before the biodegradation study was initiated. Several samples of distilled water, tap water, secondary effluent, and raw wastewater were dosed with various quantities of CMT up to 20 mg/l and assayed. Linear and consistent responses were obtained on all samples except those made up in distilled water. This unusual problem was circumvented by using tap water for reference and standard preparations. It is suspected that the erratic results obtained with distilled water were due to the absence of ions which may stabilize the colored end product.

As with many colorimetric methods occasional erratic results even on duplicate samples were experienced. Fluctuations in the measured CMT content of the same feed, and the apparent lack of significant biodegradation on CMT after ten weeks of exposure raised some doubts about the reliability of this method. A thorough check of the method, jointly by personnel from this laboratory and a visiting chemist from Monsanto Chemical Company, was made early in September 1978. A total of thirty-four standard and experimental samples were assayed independently by both

laboratory personnel. The results obtained were remarkably close to one another on all samples, thus reaffirming confidence in the method and its suitability for this study.

Interferences with the CMT analysis seldom exceeded 2 mg/l in the raw and 1.0 mg/l in the secondary effluent. These are suspected to be due to glycolic acid and acetaldehyde among many other possible interfering components found in wastewater. In view of this, coupled with experimental errors, any quantity of 2 mg/l or less should be viewed with uncertainty.

Gas Chromatographic Method

This unpublished method was developed by C. B. Warren of Monsanto Chemical Company. It is based on esterification of CMT with n-propyl alcohol and subsequent analysis by GC. A detailed description of the GC procedure including the esterification step is given in Appendix A, Part II.

Experiments with raw wastewater and secondary effluent dosed with several concentrations of CMT produced linear response. However, plots of the detector response versus CMT concentration shifted with different experiments though no interferences were noted in any raw wastewater or secondary effluent. Also, identical samples of a CMT reference standard solution analyzed routinely with authentic samples, occasionally produced inconsistent results. These anomalies are suspected to be due to an occasional incomplete esterification of the CMT. Despite these shortcomings sufficient data were gathered to indicate when biodegradation of the CMT started, and to verify the reliability of data obtained by the colorimetric method.

Comparison of Analytical Methods

A total of sixty-six parallel analyses were made by both the colorimetric and the GC methods using samples of feed and effluent of the fermenters. In forty-six of these analyses agreement within $\pm$ 25 percent was obtained by both methods; the remaining twenty analyses exceeded this limit. The seemingly wide margin of error and the high incidence of "disagreement" of results by both analytical methods are not considered excessive in view of the several critical steps in the esterification process of the gas chromatographic samples, combined with the usually large experimental errors of non-specific colorimetric methods. Results on comparative samples for an effluent from a reactor receiving synthetic wastewater and 20 mg/l CMT are shown on Figure A-1.

Repeated analyses of identical samples of 20 mg/l standard solution of CMT by the GC method often produced results far more inconsistent and erratic than comparable analyses by the colorimetric method. Consequently, the utility of the GC method was limited to checking the patterns of the GC results versus the colorimetric results. These patterns were reasonably parallel particularly once biodegradation of the CMT began to occur.

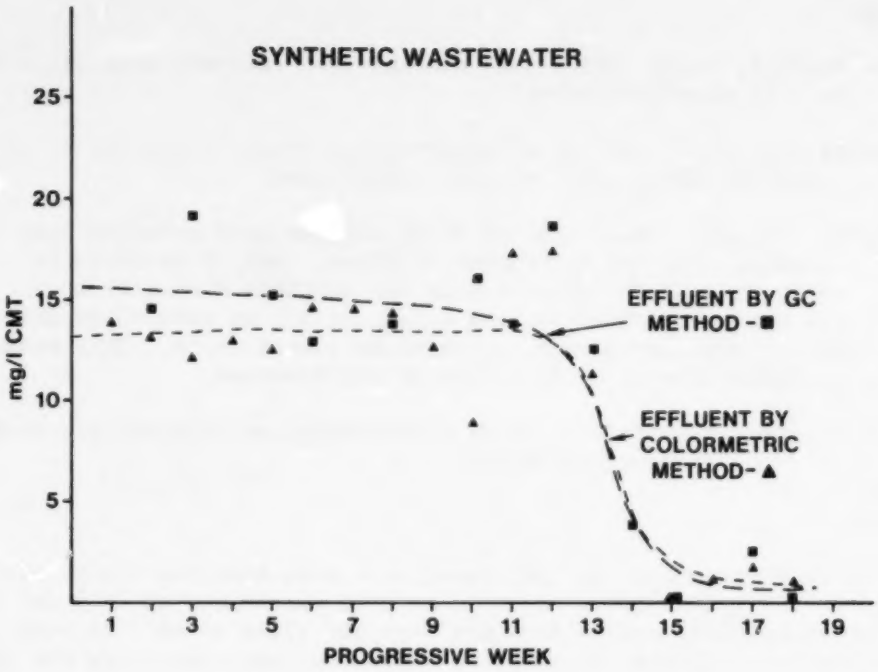

Figure A-1. Comparative Analytical Methods for CMT in Reactor Effluent

APPENDIX A, PART I

Colorimetric Assay for Carboxymethyltartronate

Reagents:

92.5% Sulfuric Acid: Add 100 ml concentrated sulfuric acid (95.5-96.5%) to 7 ml distilled water.

80% Sulfuric Acid: Add 100 ml concentrated sulfuric acid to 37 ml distilled water, cool to room temperature.

β-Napthol Reagent: Weigh out 50 mg of reagent grad β-Napthol and transfer into 100 ml volumetric flask. Add 75 ml of 92.5% sulfuric acid and allow 1 hour for complete dissolution of the β-Napthol crystals, and dilute to 100 ml with 92.5% sulfuric acid. When not in use, store in dark cool place. This reagent is stable for up to four days if refrigerated.

3:1 Hydrochloric Acid: Add 75 ml of concentrated hydrochloric acid to 25 ml of distilled water.

Procedure:

One ml sample (unfiltered) is placed in a test tube and 0.1 ml of 3:1 mixture of HCl is added. Place in an oven maintained at 105° for 18-20 hours (overnight), remove from the oven and allow to cool to room temperature. Add 1 ml of β-Napthol solution, mix vigorously for one minute on a vortex mixer and return to the same oven for one hour. Allow to cool and add exactly 3 ml of 80% sulfuric acid, mix thoroughly. Allow the sample to stand at room temperature for twenty minutes and read the absorbance at 480 nm using distilled water in the reference side of the spectrophotometer.

A reagent blank and 1 ml sample of standard solution containing 20 mg/l CMT are assayed along with the authentic samples for reference.

Calculations:

Subtract the blank absorbance from the standard and sample absorbances and proceed as follows.

$$\text{mg/l CMT in sample} = \frac{\text{corrected sample absorbance}}{\text{corrected std absorbance}} \times \text{mg/l CMT in std (20 mg/l)}$$

APPENDIX A, PART II

Gas Chromatographic Assay for Carboxymethyltartronate

Reagents:

1-propanol, trifluoracetic acid, acetyl chloride, 1,2-dichloroethane, tetracosane (C-24), Freon 113 (1,1,2-trichlorotrifluorethane, b.p. 47°C).

Solutions:

Propanol-HCl: Slowly add, with rapid stirring, 20 ml of acetyl chloride to 70 ml of ice bath cooled propanol contained in a 250 ml Erlenmeyer flask. The solution is then adjusted to 100 ml with propanol.

Tetracosane solution: A final solution of 5 mg/l of tetracosane (C-24) in 1, 2-dichlorethane is prepared from appropriate stock solutions. A more concentrated final solution will interfere with the CMT analysis. C-24 is used as a marker to identify CMT in the chromatogram.

Derivatization:

1 ml sample (unfiltered) is placed in a 150 x 15 mm test tube, freeze dried, and an internal standard of 1.0 mg of tetracosane (0.004 ml of final reagent solution) is added. 0.5 ml of trifluoracetic acid was also added to remove nitrite interference. The tube is shaken vigorously on a vortex mixer for one minute and placed in a 65°C water bath to help dissolve all of the residue. The test tube is then placed in a 70°C water bath and the trifluoracetic acid stripped off with a stream of nitrogen; 2.0 ml of propanol-HCl solution was added, warmed to 65°C and mixed vigorously on the vortex mixer for one minute, and placed in a 65°C water bath for thirty minutes to esterify the CMT. The test tube is then placed in a 70°C water bath and the propanol-HCl solution is stripped off with a stream of nitrogen. The residue is dissolved into 2 ml of Freon 113 and transferred into a 5 ml graduated centrifuge tube. One subsequent washing of the test tube with 1 ml of Freon 113 is recommended, with the addition of the wash solution into the centrifuge tube. The final volume of the Freon solution is adjusted to 1.0 ml using nitrogen to strip off the excess Freon.

A standard CMT solution containing 20 mg/l of CMT should be analyzed with the experimental samples for reference and calibration of the gas chromatograph.

Analysis:

0.005 ml samples of the final Freon solution were analyzed on a Perkin-Elmer Model 3920 gas chromatograph equipped with a dual flame ionization detector system. The column was 0.91 m x 3.2 mm stainless steel packed with Talsorb (0.65% ethylene glycol adipate on heat treated acid washed chromosorb W) available from Regis Chemical Company, Morton Grove, Illinois. The oven temperature was programmed from 80°C to 220°C at 16°C/minute. Nitrogen carrier gas was used at a flow rate of 25 ml/minute.

The CMT emerged from the column after 7.4 minutes followed by tetracosane (C-24) at 7.7 minutes. Though the retention times of the two peaks were close, their resolution was excellent as shown in Figure A-2.

For calculating sample content CMT, the peak height was compared with the corresponding peak of a standard solution processed through the entire procedure at the same time the unknowns were determined.

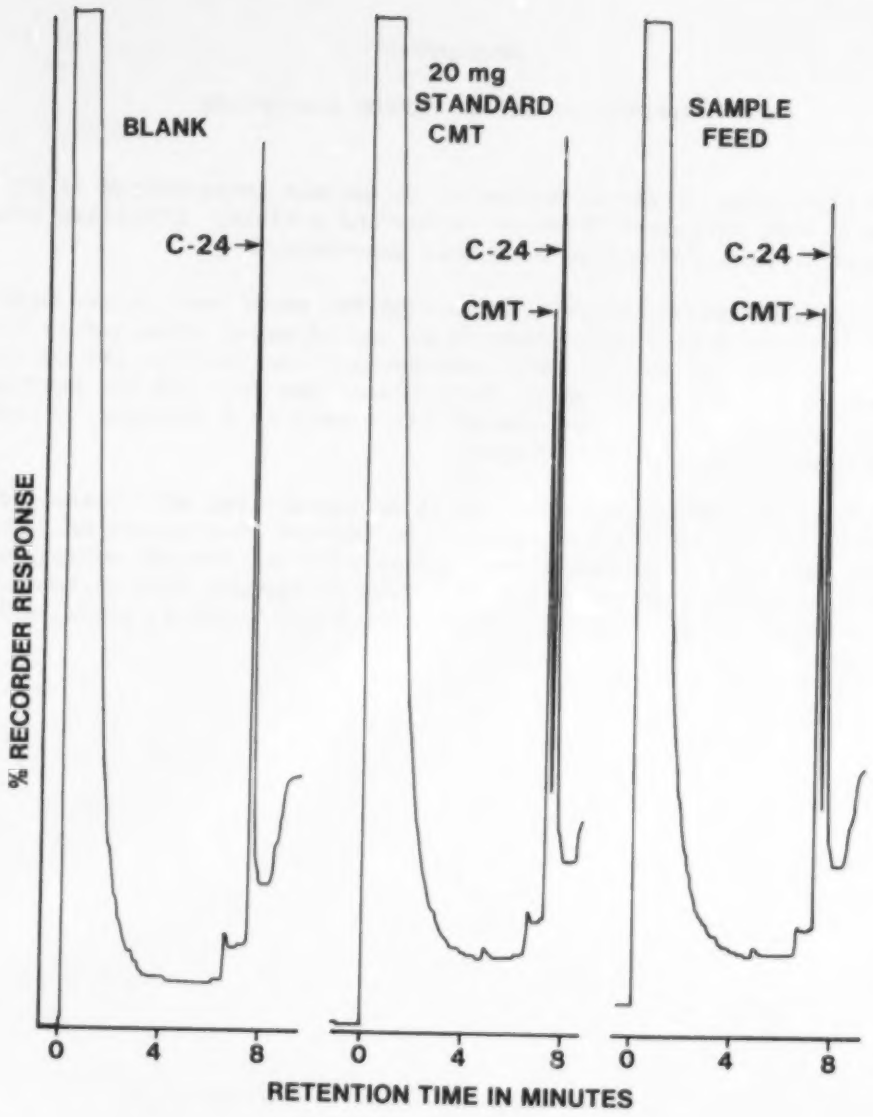

Figure A-2. Resolution of CMT by Gas Chromatography

APPENDIX B

REMOVAL OF CMT BY CARBON ADSORPTION

The adsorption of CMT by activated carbon was investigated at pH 3, 7 and 9 using five different doses of carbon and a blank. Distilled water spiked with 20 mg/l CMT was used in this experiment.

The carbon, Calgon Filtrasorb 200 (200/400 mesh) was stirred magnetically for one hour into 1 liter samples of spiked water contained in 2-liter beakers, filtered and the filtrate subsequently analyzed for CMT by colorimetric analysis. Table B-1 gives the residual CMT (C_f) and the percent removal. Figure B-1 shows the removal efficiency as a function of carbon dosage for the three pH levels tested.

Maximum CMT removal was obtained at an impractical pH 3 value. At pH 7, which would be typical for wastewater, the removal rate ranged only from 12-50% for the various carbon doses; and at pH 9 the removal efficiency declined further to a range of 15-36%. Thus it appears that carbon treatment for the removal of CMT on a full plant scale basis would be unrealistic.

TABLE B-1. Carbon Adsorption of CMT

Carbon Dose mg/l	Distilled Water With 20 mg/l CMT Added								
	pH = 3			pH = 7			pH = 9		
	C_f	$C_o - C_f$	Percent Removal	C_f	$C_o - C_f$	Percent Removal	C_f	$C_o - C_f$	Percent Removal
0	25.3	-	-	22.4	-	-	20.3	-	-
200	2.4	22.9	91	18.6	3.8	17	15.7	4.6	23
400	1.4	23.9	94	19.8	2.6	12	17.2	3.1	15
600	1.3	24.0	96	14.6	7.8	35	15.5	4.8	24
800	0.5	24.8	98	14.4	8.0	36	12.9	7.4	36
1000	0.2	25.1	99	11.3	11.1	50	15.6	4.7	27

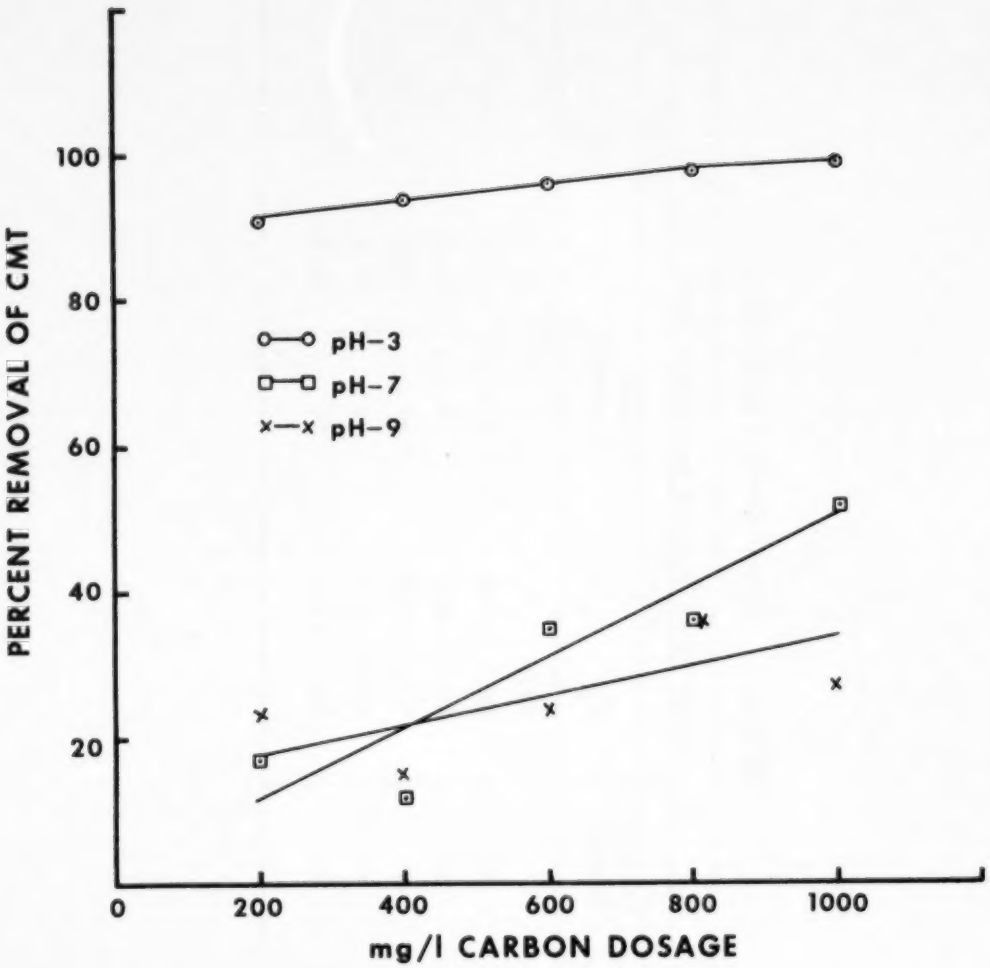

Figure B-1. Carbon Adsorption of CMT
at Various pH Values